

Application No. 10/627,253

Preliminary Amendment Dated: April 5, 2004

Reply to Notice to Comply with Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures dated February 3, 2004

Prior to examining the above-identified application, kindly amend the application as follows.

**Amendments to the Specification** begin on page 3 of this paper.

**Remarks** begin on page 5 of this paper.

**Amendments to the Specification:**

Please replace the Substitute Sequence Listing (pages 1-105) filed December 22, 2003 with the Second Substitute Sequence Listing (pages 1-91), enclosed herewith.

Please also delete the paragraph on page 2, line 24 to page 3, line 10 and replace it with the following paragraph:

In contrast to P-glycoprotein and to other members of the MRP family (MRP-4 and MRP-5), e.g. MRP-2 and MRP-1 possesses an additional N-terminal transmembrane domain (TMD0). Thus, these proteins contain two characteristic hydrophilic, cytosolic ATP-binding domains (NBD's) and 3 hydrophobic transmembrane domains, which include totally 17 transmembrane segments. This is designated as TMD0(TMD-ABC)<sub>2</sub> arrangement (Klein et al. 1999, *Biochimica et Biophysica Acta* 1461: 237-262). The NBD's are characterized by two sequence motifs, designated „Walker A“ and „Walker B“. Mutational analysis of a number of ABC proteins indicates that these two regions are critical for ATPase function (Walker et al. 1982, *EMBO J.* 1: 945-951; Schneider et al. 1998, *FEMS Microbiol. Rev.* 22: 1-20). Within the Walker A motif there exists a conserved lysine residue (GX<sub>4</sub>GKS/T), which is essential in both nucleotide binding domains for full transport function. This is consistent with the role of this consensus sequence as the amino acid acceptor site of the phosphoryl moiety of the nucleotide. In addition, ABC transporters possess a characteristic conserved „active transport family“ signature (or „C“) motif encompassing 14 amino acids (LSSGGQX<sub>3</sub>RHydXHydA)(**SEQ ID NO: 406**). This region is located between the Walker A and B motifs. A possible significance of this motif referring to the binding and hydrolysis of nucleotide could be deduced from the observation, that it is highly conserved in NBD1, but not in

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NBD2 of the MRP-related proteins. This is in contrast to observations, which point to a invariant nature of this motif in NBD1 and NBD2 in P-glycoproteins (Cole and Deeley 1998, BioEssays 20: 931-940).